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A retrospective study of bacterial pathogens in an equine hospital (1988-2014)

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Abstract EN

Knowledge about local pathogen prevalence is important to guide initial antimicrobial therapy. The aim of this study was to describe longitudinal occurrence of bacterial pathogens from hospitalized horses at the University of Zurich between 1988 and 2014. Positive culture results were retrospectively analyzed. Isolates were grouped based on sample site, diagnosis, and year of isolation. Frequency of bacterial isolates over time was compared using the Chi-square test. *Escherichia coli* (299/1723, 17%)*, Streptococcus equi* ssp*. zooepidemicus* (295/1723, 17%), and coagulase-positive staphylococci (196/1723, 11%) were the most common pathogens. Anaerobes were common in dental (18/66, 27%), peritoneal (6/43, 14%), and soft tissue infections (28/208, 13%). Occurrence of mixed infections was common in skin and dental infections (24/55, 44% and 17/43, 40%, respectively). Frequently isolated bacteria included in most organ systems Gram-positives and Gram-negatives, supporting the need for initial broad-spectrum antimicrobial therapy until culture and susceptibility results become available.

(horse, hospitalized patients, bacteria, retrospective long-term analysis)

Zusammenfassung DE

Kenntnisse über lokal vorkommende pathogene Bakterien sind wichtig, um eine gezielte antibiotische Therapie einleiten zu können. Das Ziel der Studie war es, die häufigsten Infektionserreger und deren zeitliche Veränderungen bei Pferden am Tierspital Zürich zwischen 1988 und 2014 zu beschreiben. Alle positiven Kulturergebnisse wurden retrospektiv analysiert. Die Isolate wurden anhand ihrer Lokalisation, Diagnose und Jahr der Isolation eingeteilt. Die Häufigkeit verschiedener bakterieller Isolate in verschiedenen Zeitphasen wurde mittels Chi-Square Test verglichen. *Escherichia coli* (299/1723, 17%)*, Streptococcus equi* ssp*. zooepidemicus* (295/1723, 17%) und koagulasepositive Staphylokokken (196/1723, 11%) waren die insgesamt am häufigsten nachgewiesenen Bakterien. Obligat anaerobe Bakterien wurden vorwiegend bei Zahninfektionen (18/66, 27%), Peritonitis (6/43, 14%) und Weichteilinfektionen (28/208, 13%) isoliert. Von 346/1281 (27%) Proben wurden Mischkulturen isoliert, besonders häufig bei Haut- und Zahninfektionen (24/55, 44% und 17/43, 40%). Zu den am häufigsten isolierten Keimen gehörten in den meisten Organsystemen sowohl Gram-positive als auch Gram-negative Erreger. Daher sollte die Wahl des Antibiotikums, bis zum Vorliegen der Kulturresultate und Antibiogramme auf ein Medikament mit breitem Wirkspektrum fallen.

(Pferd, hospitalisierte Patienten, Bakterien, retrospektive Langzeitanalyse)

A retrospective study of bacterial pathogens in an equine hospital (1988–2014)

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Abstract

Knowledge about local pathogen prevalence is important for guiding initial antimicrobial therapy. The aim of this study was to describe the longitudinal occurrence of bacterial pathogens in hospitalized horses at the University of Zurich between 1988 and 2014. Positive culture results were retrospectively analyzed. Isolates were grouped based on sample site, diagnosis, and year of isolation. The frequency of bacterial isolates over time was compared using the Chi-Square test. *Escherichia coli* (299/1,723, 17%)*, Streptococcus equi* ssp. *zooepidemicus* (295/1,723, 17%), and coagulase-positive staphylococci (196/1,723, 11%) were the most common pathogens. Anaerobes were common in dental (18/66, 27%), peritoneal (6/43, 14%) and soft tissue infections (28/208, 13%). Mixed infections were common in skin and dental infections (24/55, 44% and 17/43, 40%, respectively). Frequently isolated bacteria in most organ systems included Gram-positives and Gram-negatives, supporting the need for initial broad-spectrum antimicrobial therapy until culture and susceptibility results become available.

Introduction

Bacterial infections are a major cause of morbidity and mortality in horses. The identification of pathogenic bacteria in disease processes is important for understanding pathogenesis and optimizing therapy (Sellon and Long, 2013). While clinical findings often allow a presumptive diagnosis of bacterial infection, confirmation and characterization of the causative agent is achieved through bacteriological culture and susceptibility testing (Morley et al., 2005). However, culture results are often not available for up to 48 hours, and an empiric choice of antimicrobial treatment has to be made (Wilson, 2001). To guide the choice of initial antimicrobial therapy, knowledge of common pathogens in affected organ systems as well as of antimicrobial resistance of common pathogens is important (Wilson, 2001). There are studies available describing bacterial isolates from equine infections, however, only few of them are recent (Hirsh and Jang, 1987; Lavoie et al., 1991; Sweeney et al., 1991; Hawkins et al., 1993; Racklyeft and Love, 2000; Clark et al., 2008; Panchaud et al., 2010; Theelen et al., 2014). Additionally, bacterial prevalence can differ geographically; therefore, surveillance of bacterial isolates is recommended on a local level (Morley et al., 2005; Traub-Dargatz and Dargatz, 2009). There is currently little data available of common bacterial pathogens in horses in Switzerland.

In recent years, antimicrobial drug resistance has become an emerging problem in human and veterinary medicine (Weese, 2009). The acquisition of resistance against antimicrobials permits survival of particular bacterial strains, and thus changes in susceptibility profiles are able to shift the prevalence of pathogens. Therefore, surveillance of changes in prevalence is recommended (Morley et al., 2005; Traub-Dargatz and Dargatz, 2009). The objective of this study was to retrospectively describe occurrence and longitudinal development of bacterial pathogens isolated from equine infections at the University of Zurich between 1988 and 2014.

Material and Methods

Data collection

The database of the Institute of Veterinary Bacteriology and the Equine Hospital of the University of Zurich were retrospectively searched for bacterial isolates cultured from horses presented to the Equine Hospital of the University of Zurich. The information obtained included date of collection, organ system affected, bacterial strain isolated and number of pathogenic bacteria per sample.

Classification

Infections were classified into 12 groups based on the origin of the samples. Respiratory tract infections included pathogens isolated from the upper and lower airways, including pleural involvement. Implant infections (orthopedic), infections of incisions and injection sites (soft tissue) and infections after dental procedures (sinus) were grouped as post-procedural infections. Synovial infections, osteitis and hoof abscesses were combined to musculoskeletal infections. Soft tissue infections included external abscesses, wounds, cellulitis, infected hematomas and myositis. Isolates from the uterus, vagina, lochia, prepuce and samples from mastitis were summarized as reproductive infections. Urinary tract infections included upper and lower urinary tract infection and omphalitis. Dental infections were sampled from horses with clinical appearance of an alveolar periostitis, dental sinusitis or osteitis due to dental disease. Nervous system infections contained all isolates from cerebrospinal fluid. Bacterial isolates from undetermined sites and samples collected from lymph nodes, ear swabs, salivary glands, neoplasia and liver biopsies were classified as ‘others’. Bacteria isolated from fecal samples were excluded. Isolates considered as non-pathogenic or contamination were excluded based on information on bacterial species, origin of sample, quantity of growth, the occurrence of mixed cultures and clinical findings. If opportunistic pathogens were isolated, they were only included if a role as causative pathogen seemed reasonable based on clinical findings. When mixed infections occurred in one sample, all isolates were included. When a bacterial strain was isolated more than once from the same site in a horse within six months, the isolate was only counted once in the study (persistent infection).

Period of time

The available data was divided into four phases: 1988–2002 (A1), 2003–2006 (A2), 2007–2010 (B1) and 2011–2014 (B2). The time spans were chosen to reflect major changes in antimicrobial treatment at the Equine Hospital. From 1988 to 2006 (A1 and A2), the main first-line antibiotic treatment choice was penicillin, normally combined with the aminoglycoside gentamicin. Due to a perceived increase of resistance against gentamicin, cefquinome was used more frequently as a first-line antibiotic agent between 2007 and 2014 (B1 and B2).

Culture methods

Depending on the isolation site, samples were streaked on standard agars (Columbia blood agar with sheep blood, Gassner agar, Columbia CNA agar, Chocolate agar with vitox, Oxoid AG, Pratteln, Switzerland) and incubated at 37°C for 24–48 hours under aerobic conditions. For culture of obligate anaerobes, samples were streaked on Columbia blood agar with sheep blood and Schaedler KV agar (Oxoid AG, Pratteln, Switzerland) and incubated at 37°C under anaerobic conditions for 48–72 hours. The samples were investigated by standard bacteriological procedures using gram-staining, catalase test, oxidase test, oxidation-fermentation test, agglutination test to check coagulase activity, agglutination test to classify the Lancefield groups of *Streptococcus* spp. and growth performance on different agar (Markey, 2013). Identification to species level was done using the API® test system (1988–2011) or the VITEK® 2 compact system (2012–2014) (both bioMérieux, Marcy l`Etoile, France) using the appropriate API® strips or VITEK® cards according to the manufacturer's instructions.

Statistical analysis

To compare frequency distributions between different phases, the Chi-Square test or Fisher exact test was used. Statistical analysis was performed using commercial software (GraphPad Prism 6). The Bonferroni correction for multiple comparisons was applied and results were considered statistically significant if $p<0.0125$.

Results

The database yielded 1,723 bacterial pathogens isolated from 1,281 samples from horses between 1988 and 2014. During phase A1 (1988–2002) 258/1,723 (15%) isolates were obtained, during phase A2 (2003–2006) 481/1,723 (28%), during phase B1 (2007–2010) 445/1,723 (26%) and during phase B2 (2011–2014) 539/1,723 (31%). The majority of isolates was obtained from respiratory (397/1,723, 23%), post-procedural (386/1,723, 22%) and soft tissue infections (208/1,723, 12%). An overview of the origin of samples is shown in Table 1. In 940/1,281 (73%) samples a single bacterial species was identified, whereas in 346/1,281 (27%) samples mixed infections (2–6 isolates) occurred. In 206/346 (60%) mixed infections simultaneous infection with Gram-positive and Gram-negative bacteria was present. The occurrence of mixed infections varied between organ systems (Fig. 1).

The most commonly isolated bacteria were *Escherichia* (*E.*) *coli* (299/1,723, 17%), *Streptococcus* (*S.*) *equi* ssp. *zooepidemicus* (295/1,723, 17%) and coagulase-positive staphylococci (196/1,723, 11%). All isolated bacterial pathogens are listed in Table 2. Coagulase-positive staphylococci were grouped together, whereof 136/196 (69%) were *Staphylococcus aureus*. Of *Acinetobacter* spp. 31/70 (44%) were further classified into *A. baumannii*. Aerobic bacteria dominated with 1,599/1,723 (93%) isolates: 859/1,599 (54%) were Gram-positive and 740/1,599 (46%) Gram-negative. Obligate anaerobic bacteria represented 124/1,723 (7%) isolates from 113 samples: 22/124 (18%) were Gram-positive, 67/124 (54%) were Gram-negative and 35/124 (28%) were not further classified. Mixed aerobe and anaerobe infections were present in 62/113 (55%) of samples. Most common obligate anaerobes were *Bacteroides* spp. (37/124, 30%), *Clostridium* spp. (18/124, 15%) and *Fusobacterium* spp. (18/124, 15%).

Statistical analysis was performed for bacterial strains isolated at least 10 times (>0.5%). Significant changes over time were identified for anaerobes ($p<0.0001$), coagulase-negative staphylococci ($p<0.0001$), *Enterococcus* spp. ($p<0.0001$), *Pseudomonas* (*P.*) *aeruginosa* ($p<0.0001$), *Acinetobacter* spp. ($p=0.0079$), *Pasteurella* spp. ($p=0.0007$), *Proteus* spp. ($p=0.0065$), *Actinobacillus* spp. ($p=0.002$), *Pantoea agglomerans* ($p<0.0001$), *S. dysgalactiae* ssp. *equisimilis* ($p=0.0074$), and *Trueperella* (formerly *Arcanobacterium*) *pyogenes* ($p=0.0046$). Both increases and decreases over time were observed (Fig. 2).

The distribution of the most common bacterial strains by organ system is shown in Table 3. Due to the small numbers of bacterial strains in several organ systems, longitudinal analysis of bacterial isolates was only performed for respiratory, post-procedural, soft tissue, and musculoskeletal infections. Significant changes over time were seen in respiratory and post-procedural infections (Fig. 3).

Discussion

This study showed that *E. coli, S. equi* ssp. *zooepidemicus* and coagulase-positive staphylococci were the 3 most commonly isolated equine pathogens. In most organ systems Gram-negative as well as Gram-positive bacteria were common. These results agree with previous studies reporting *E. coli* and *S. equi* ssp. *zooepidemicus* as the most commonly isolated equine pathogens (Lavoie et al., 1991; Clark et al., 2008; Panchaud et al., 2010). Similar to an earlier study in another region of Switzerland (Panchaud et al., 2010), coagulase-positive staphylococci, including *S. aureus,* were particularly numerous, while *Actinobacillus* spp., an important pathogen in other countries, was uncommon (Lavoie et al., 1991; Clark et al., 2008). Differing bacterial prevalence between geographic regions has previously been described (Sellon and Long, 2013) and is likely due to differing environments, prophylactic and therapeutic measures, eradication strategies, biosecurity and antimicrobial use. This further highlights the need for prevalence studies in different regions.

S. equi ssp. *equi* is a common respiratory pathogens in Switzerland (Panchaud et al., 2010), showing that strangles is still widely prevalent in the Swiss horse population. *Rhodococcus equi,* an important respiratory pathogen of foals in geographically close regions (Venner et al., 2013), was only isolated from 5/397 (1%) samples. Anaerobes, often involved in cases of pleuropneumonia (Sweeney et al., 1991), were rarely isolated from respiratory infections, which corresponds with the fact that pleuropneumonia is an uncommon disease at our hospital. Frequent isolates from post-procedural infections in our and other studies (Clark et al., 2008; Taylor et al., 2010; Torfs et al., 2010) included *E. coli, Staphylococcus* spp., *Enterococcus* spp., *P. aeruginosa,* and *Acinetobacter* spp.. These species are known for their ability to develop high rates of antimicrobial resistance, and multidrug-resistant isolates are reported to cause incisional and implant infections (Weese, 2009). Thus, treatment in post-procedural infections should always be guided by a susceptibility profile. In peritonitis cases, isolation of *E. coli* and anaerobes was common and in accordance with previous studies (Hirsh and Jang, 1987; Lavoie et al., 1991; Hawkins et al., 1993; Southwood and Russell, 2007). Only a single case of unclassified *Actinobacillus* spp. occurred, while this pathogen is an important cause of equine peritonitis in Australia and America (Matthews et al., 2001; Southwood and Russell, 2007). In neonatal sepsis, *Enterobacteriaceae* and other Gram-negatives are the most common isolates (Lavoie et al., 1991; Clark et al., 2008; Theelen et al., 2014). However, an increase in Gram-positive isolates, especially *Enterococcus* spp., has

been reported recently (Theelen et al., 2014). Sepsis cases in our study showed a similar spectrum, but the case numbers were too low for longitudinal analysis. Nervous system infection in foals is usually a sequel to sepsis (Tyler et al., 1993; Pusterla et al., 2007; Hepworth et al., 2014). In this study *E. coli* was isolated from the cerebrospinal fluid of a foal with sepsis and a corresponding positive blood culture. In contrast, little is known about bacterial agents causing meningoencephalitis in adult horses. Only 2 cases of adult horses with clinical signs of meningoencephalitis and a positive culture result for *Acinetobacter* spp. and *Enterococcus* spp. respectively occurred over the study period. Prediction of the etiologic pathogen is therefore difficult in these cases, highlighting the need for bacteriologic culture.

As reported before (Lavoie et al., 1991), most samples (935/1,281, 73%) yielded pure cultures of one sole bacterial pathogen. Mixed infections were particularly common in organs colonized with a large number of commensal bacteria like the skin or the oral cavity.

While the occurrence of main pathogens remained stable, some bacterial strains emerged, and others were less commonly isolated over the last years, displaying a dynamic interaction between horse population and pathogens adapting to their environment. Antimicrobial therapy has an impact on bacterial prevalence, as it allows bacteria to grow which are intrinsically resistant or able to develop resistance to the used antimicrobial drug (Morley et al., 2005; Dunowska et al., 2006). Some antimicrobial classes have a greater potential to promote selection of resistant isolates than others, as shown for cephalosporins in equine fecal *E. coli* (Dunowska et al., 2006). The change of first-line antibiotics from penicillin/gentamicin to cefquinome in 2007 could have influenced the bacterial spectrum. The increased isolation rates of *Enterococcus* spp., *Acinetobacter* spp., and coagulase-negative staphylococci possibly reflect their ability to adapt quickly and develop resistance to several antimicrobial drugs. In addition, *Enterococcus* spp. are intrinsically resistant to all cephalosporins (Weese, 2009), which could further explain their increasing occurrence during the last years. Multidrug-resistant enterococci (primarily *E. faecalis* and *E. faecium*), *Acinetobacter* spp. (especially *A. baumannii*), and coagulase-negative staphylococci (especially methicillin-resistant staphylococci) are increasingly important nosocomial pathogens in humans (Weese, 2009; Becker et al., 2014). Little is known about such infections in horses, but their increasing occurrence seen in hospitalized horses in this study is alarming.

One limitation of this study is its retrospective nature. Changes in laboratory techniques, clinical diagnostics, and knowledge of causative organisms occurred over the time investigated. Awareness of important isolates and new possibilities of classifying them may increase the number of positive results without a real increase in their prevalence. Furthermore, a true prevalence for each bacterial pathogen could not be calculated, because some horses with clinical signs of an infection might not have had a culture taken and samples revealing no growth were excluded.

Care should be taken when extrapolating the results to private practice, as all our samples were taken from hospitalized horses and probably many of them were already pretreated with antibiotics.

Conclusions

Gram-positive as well as Gram-negative bacteria were common isolates in almost all organ systems. Furthermore, in dental, peritoneal and soft tissue infections anaerobic bacteria should be considered. In conclusion, initial broad-spectrum antimicrobial therapy is generally necessary until bacteriological culture and susceptibility testing results become available. However, clinical diagnosis may allow targeted antimicrobial therapy in some cases (e.g. strangles). Longitudinal changes reflect a dynamic interaction between pathogens, environment and host. Antimicrobial therapy does influence bacterial prevalence as it selects for isolates resistant to the used antibiotic. Monitoring of bacterial pathogens in horses should be continued and susceptibility testing data of reported pathogens analyzed.

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Figures

Figure 1: Distribution of equine samples (n=1,281) with a single species of pathogenic bacteria and proportion of mixed infections by organ system isolated in Zurich between 1988 and 2014.

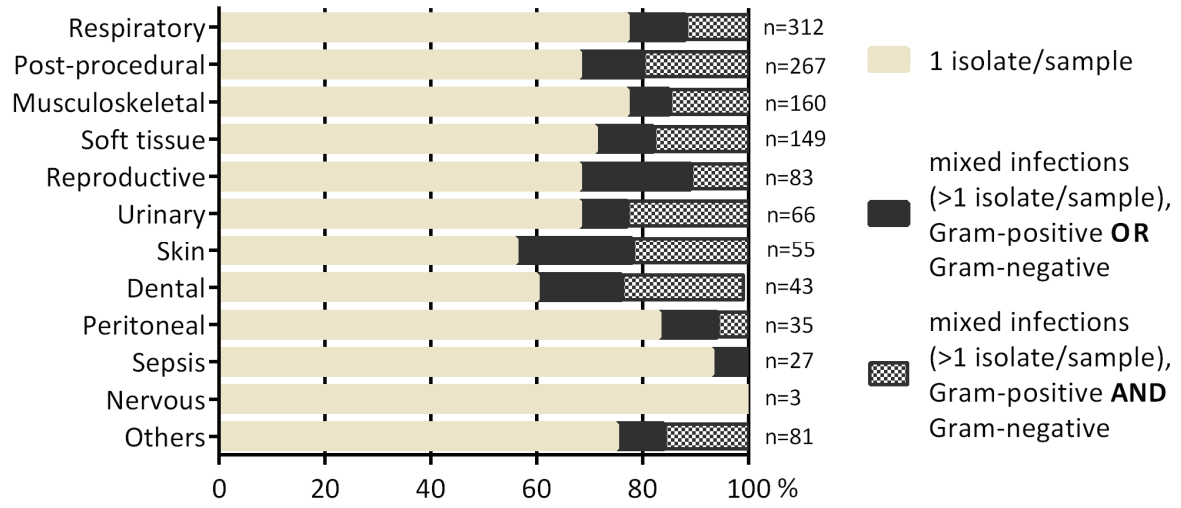


Figure 2: Changes in frequency of pathogenic bacterial strains over time isolated from horses between 1988 and 2014 (n=1,723).

* indicates a significant change over time, different letters show significant difference between two groups (p <0.0125)

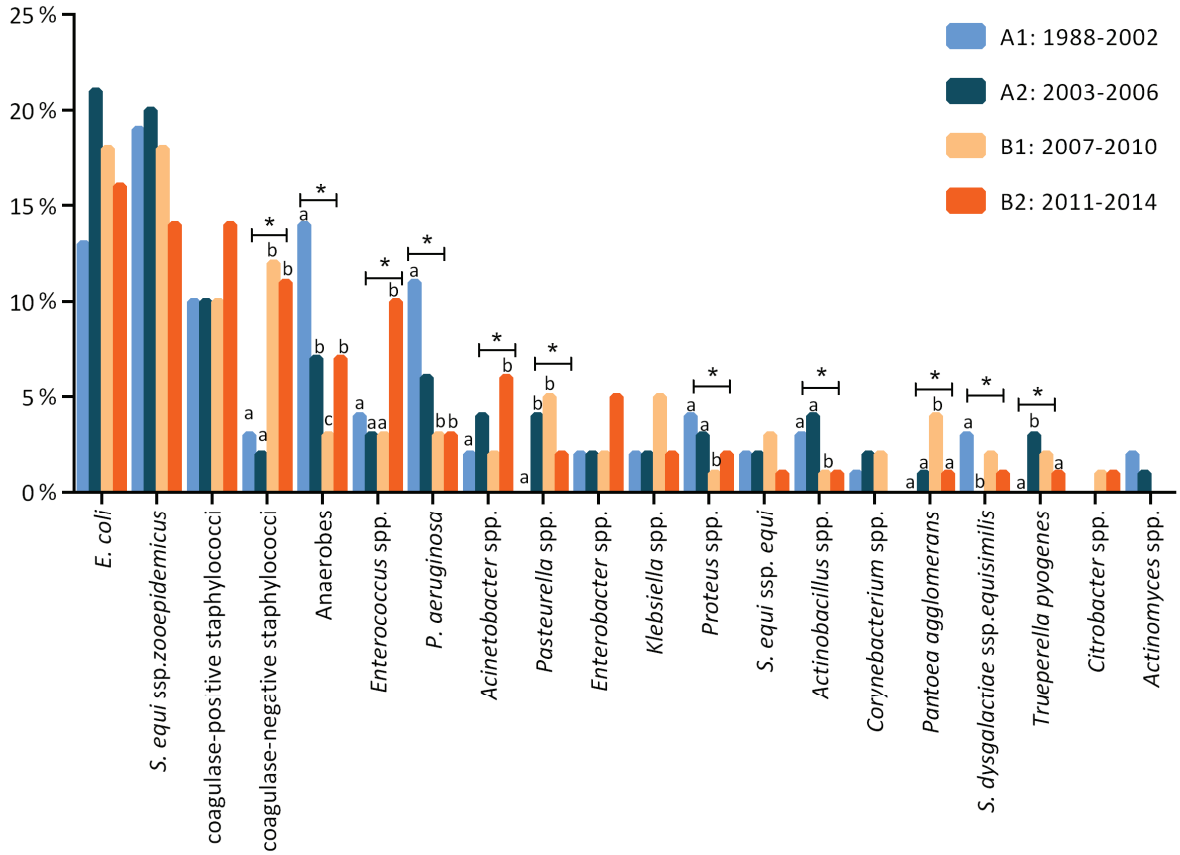
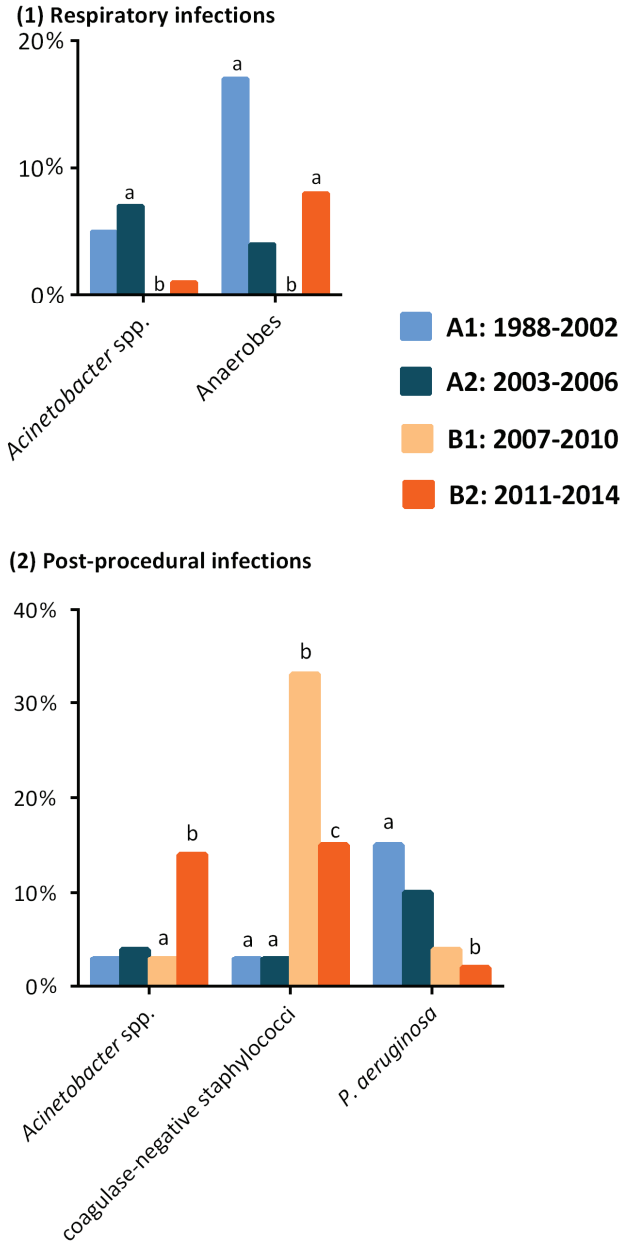


Figure 3: Bacterial strains with significant changes over time cultured from equine (1) respiratory (n=397) and (2) post-procedural infections (n=386) in Zurich between 1988 and 2014.

Different letters show significant difference between two groups ($p < 0.0125$)



Tables

Table 1: Origin of bacterial pathogens isolated from horses in Zurich between 1988 and 2014.

Organ system and diagnosis	Number of isolates per organ system (%)	Number of isolates per diagnosis (%)
Respiratory	397 (23%)	
Lower airway infection		256 (64%)
Upper airway infection		141 (36%)
Post-procedural	386 (22%)	
Post-procedural (soft tissue)		267 (69%)
Post-procedural (orthopedic)		106 (28%)
Post-procedural (sinus)		13 (3%)
Soft tissue	208 (12%)	
External abscess		110 (53%)
Soft tissue wound		52 (25%)
Cellulitis		41 (20%)
Infected hematoma		4 (2%)
Myositis		1 (<1%)
Musculoskeletal	207 (12%)	
Synovial infection		96 (46%)
Osteitis		72 (35%)
Hoof abscess		39 (19%)
Reproductive	99 (6%)	
Genital tract infection		97 (98%)
Mastitis		2 (2%)
Urinary	95 (6%)	
Urinary tract infection		67 (71%)
Omphalitis		27 (28%)
Renal abscess		1 (1%)
Skin	87 (5%)	
Dental	66 (4%)	
Peritoneal	43 (3%)	
Sepsis	27 (2%)	
Nervous system	3 (<1%)	
Others	108 (6%)	
Total	1723 (100%)	

Table 2: Bacterial isolates from horses in Zurich between 1988 and 2014.

Bacterial strains with less than 10 counts (<0.5%) were grouped in 'others'

* Formerly *Arcanobacterium pyogenes*

Bacterial isolate	n (%)
E. coli	299 (17.4%)
S. equi ssp. *zooepidemicus*	295 (17.1%)
coagulase-positive staphylococci	196 (11.4%)
coagulase-negative staphylococci	133 (7.7%)
Anaerobes	124 (7.2%)
Enterococcus spp.	94 (5.5%)
P. aeruginosa	87 (5.1%)
Acinetobacter spp.	70 (4.1%)
Pasteurella spp.	55 (3.2%)
Enterobacter spp.	52 (3.0%)
Klebsiella spp.	46 (2.7%)
Proteus spp.	40 (2.3%)
S. equi ssp. *equi*	35 (2.0%)
Actinobacillus spp.	32 (1.9%)
Corynebacterium spp.	26 (1.5%)
Pantoea agglomerans	25 (1.5%)
S. dysgalactiae ssp. *equisimilis*	24 (1.4%)
*Trueperella pyogenes**	23 (1.3%)
Citrobacter spp.	14 (0.8%)
Actinomyces spp.	11 (0.6%)
Others:	<10 (<0.5%)
Bordetella bronchiseptica, *Rhodococcus equi*, *Dermatophilus congolensis*, *Stenotrophomonas maltophilia*, *Nocardia* spp., *S. canis*, *S. uberis*, *Aeromonas* spp., *Mannheimia haemolytica*, *Neisseria* spp.	
Total	1723 (100%)

Table 3: Distribution of bacterial isolates (n=1,723) from horses in Zurich between 1988 and 2014 by organ system of origin.

Pathogenic bacteria with an occurrence of >5% isolated from the organ system are shown.

Origin of samples	Total number of isolates	Bacterial strain	n (%)
Respiratory	n=397	*S. equi* ssp. *zooepidemicus*	99 (25%)
		E. coli	58 (15%)
		S. equi ssp. *equi*	33 (8%)
		Pasteurella spp.	29 (7%)
Post-procedural	n=386	*E. coli*	83 (22%)
		coagulase-positive staphylococci	55 (14%)
		coagulase-negative staphylococci	51 (13%)
		Enterococcus spp.	30 (8%)
		P. aeruginosa	28 (7%)
		S. equi ssp. *zooepidemicus*	27 (7%)
		Acinetobacter spp.	26 (7%)
		Anaerobes	26 (7%)
Soft tissue	n=208	*S. equi* ssp. *zooepidemicus*	37 (18%)
		coagulase-positive staphylococci	36 (17%)
		Anaerobes	28 (13%)
		E. coli	21 (10%)
		Acinetobacter spp.	13 (6%)
		coagulase-negative staphylococci	12 (6%)
Musculoskeletal	n=207	*S. equi* ssp. *zooepidemicus*	35 (17%)
		coagulase-positive staphylococci	33 (16%)
		E. coli	30 (15%)
		coagulase-negative staphylococci	20 (10%)
		Anaerobes	14 (7%)
		P. aeruginosa	13 (6%)
		Proteus spp.	13 (6%)
		Enterococcus spp.	13 (6%)
Reproductive	n=99	*S. equi* ssp. *zooepidemicus*	35 (35%)
		E. coli	31 (31%)
		Enterobacter spp.	6 (6%)
Urinary	n=95	*E. coli*	30 (32%)
		Enterococcus spp.	16 (17%)
		S. equi ssp. *zooepidemicus*	12 (13%)
		coagulase-negative staphylococci	8 (8%)
		coagulase-positive staphylococci	6 (6%)
		Klebsiella spp.	6 (6%)
Skin	n=87	coagulase-positive staphylococci	29 (33%)
		coagulase-negative staphylococci	10 (11%)
		P. aeruginosa	10 (11%)
		S. equi ssp. *zooepidemicus*	8 (9%)
		Dermatophilus congolensis	5 (6%)
		Pantoea agglomerans	5 (6%)
Dental	n=66	Anaerobes	18 (27%)

		E. coli	13 (20%)
		S. equi ssp. *zooepidemicus*	10 (15%)
		P. aeruginosa	5 (8%)
		Enterococcus spp.	5 (8%)
Peritoneal	n=43	*E. coli*	13 (30%)
		Anaerobes	6 (14%)
		S. equi ssp. *zooepidemicus*	5 (12%)
		Enterococcus spp.	5 (13%)
		coagulase-positive staphylococci	3 (7%)
Sepsis	n=27	*E. coli*	8 (30%)
		coagulase-negative staphylococci	5 (19%)
		Pasteurella spp.	4 (15%)
		Actinobacillus spp.	2 (7%)
		Klebsiella spp.	2 (7%)
Nervous system	n=3	*Acinetobacter spp.*	1 (33%)
		E. coli	1 (33%)
		Enterococcus spp.	1 (33%)
Others	n=105	*S. equi* ssp. *zooepidemicus*	27 (26%)
		Actinobacillus spp.	11 (10%)
		coagulase-positive staphylococci	9 (9%)
		E. coli	9 (9%)
		Pasteurella spp.	8 (8%)
		P. aeruginosa	7(7%)
		Anaerobes	6 (6%)

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